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The Silvered Leaf Monkey (*Presbytis cristata*) as a Model for Human Bancroftian Filariasis.

James R Campbell, Harijani A Marwoto, Soekartono Tirtokusumo, Sofyan Masbar, Jan T Rusch, Purnomo, and Bambang Trenggono

The silvered leaf monkey, *Presbytis cristata*, (Ceropithecidae:Colobinae) is the first animal other than man that has been shown capable of readily supporting the complete development of the human filarial parasite *Wuchereria bancrofti* (1,2). Attempts to infect other primates (*Macaca*) resulted in patent infections, but often required prior immunosuppression of the host. In all cases microfilarial densities were low, and periodicity could not be determined (3). Attempts to produce patent infections in smaller laboratory animals have been uniformly unsuccessful (4,5). Since *P. cristata* readily develop patent *W. bancrofti* infections without prior host immunosuppression, and because this particular primate species is abundant in Indonesia, it was chosen as a model for detailed studies of bancroftian filariasis.

To be useful as an experimental model for studying bancroftian filariasis, wild caught *P. cristata* first must be thoroughly characterized by physical, hematological and parasitological parameters. This study reports such baseline data obtained from a large sample of *P. cristata*.

A total of 169 *P. cristata* from Lampung, South Sumatera and Banten, West Java, Indonesia, were used in the study. The animals were caught in the wild and brought to a primate research facility in Jakarta, Indonesia, within 2-12 days after capture. Within 24 hours' after arrival, each animal was restrained with an intramuscular injection of ketamine HCl (Vetalar, Parke-Davis, Morris Plains, NJ, 07950), 10 mg/kg body weight, and given the following examinations:(a) Thick and thin blood smears for malaria examination and a 30 µl thick smear for filiaria examination were prepared and stained with Giemsa. In addition 1cc of heparinized blood drawn between 2200-0200 hours was filtered through a 3 µm nucleopore filter, which was stained and examined for microfilariae. Another thin blood smear was stained with Wright-Giemsa and used for differential count. Hematocrit was determined by the micro-hematocrit method, hemoglobin was determined by the acid-hematin test and total white blood cell counts were made using a Neubauer hemacytometer.

(b) Physical examinations were conducted to determine sex, weight, dentition and general physical condition, and any current or old injuries were noted.

(c) Each animal was given a tuberculosis skin test in the right eyelid consisting of an intradermal injection of 0.1cc of tuberculin (Mammalian, human isolates, Burroughs Wellcome, Kansas City, MO, 64161). Skin test were repeated at 2 weeks and at 4 weeks after arrival.

(d) Feces were collected, placed in formalin and in polyvinyl alcohol (PVA) and examined for intestinal parasites. Rectal swabs were taken, placed in Amis transport media and examined within 12 hours for bacterial pathogens.

(e) Animals were placed on white paper and their entire body was thoroughly brushed with a stiff brush to dislodge ectoparasites. Further careful examinations of the skin and the ears were conducted to locate ectoparasites.

Of the 169 animals in the study, 137 (81%) were female and 32 (19%) were male. Female monkeys averaged 4.5 ± 1.3 kg (range 1.6 – 6.7 kg) and males averaged 4.5 ± 1.8 kg (range 1.6 – 8.2 kg) in body weight. Blood parameters determined for these animals are summarized in Table 1.

Examination of the monkey's blood and feces revealed a variety of naturally acquired parasitic infections, as shown in Table 2. Amoebae found in stools included *E. polecki* and *E. hartmanni*, but no *E. histolytica* was found. In addition, in several animals *Oesophagostomum* sp. was found at necropsy in nodules on the serosal surface of the large and small intestines.

Bacteriological findings revealed a very low prevalence of intestinal pathogens, as shown in Table 3. All animals were negative on three successive skin tests for tuberculosis. No ectoparasites were found on any of the animals.

Table 1 Baseline hematogical parameters in wild caught *Presbytis cristata*

parameter	n	X ± S.D.	range
Hemoglobin (g/dl)	169	9.6 ± 1.4	4.0 – 15.0
Hematocrit (%)	169	35 ± 4.6	18 – 48
WBC mm^3	164	8155 ± 3347	2100 – 18,600
Neutrophils (%)	93	50 ± 21	4 – 94
Lymphocytes (%)	93	48 ± 21	6 – 96
Eosinophils (%)	93	1 ± 5	0 – 13
Monocytes (%)	93	< 1	0 – 3
Basophils (%)	93	< 1	0 – 2

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Table 2 Naturally acquired parasitic infections in *Presbytis cristata*

species	number positive/number examined (%)
Plasmodium knowlesi (blood stages)	25/149 (16.8)
Brugia malayi (microfilariae)	7/132 (5.3)
Trichuris trichiura (ova)	123/158 (77.8)
Strongyloides sp. (larvae)	30/158 (19.0)
Ancylostomatids (ova)	13/158 (8.2)
Entamoeba sp (cysts)	49/158 (31.0)
Giardia lamblia (cysts)	3/158 (1.9)

Table 3 Bacteriological culture results from rectal swabs of wild caught *Presbytis cristata*

species	number positive/number examined (%)[a]
Salmonella group B[b]	8/180 (4.4)
Salmonella group E[c]	2/180 (1.1)
Vibrio cholerae non-O1[d]	1/180 (0.6)

[a]Data represent the number of animals with rectal swabs culture-positive for organisms, versus total number of animals examined. Data are included from 11 additional animals that died before complete examinations could be performed.
[b]Seven of these isolates were tetracycline resistant *in vitro*, one was tetracycline and ampicillin resistant.
[c]Both isolates were tetracycline resistant.
[d]Isolate was colistin sulfate resistant.

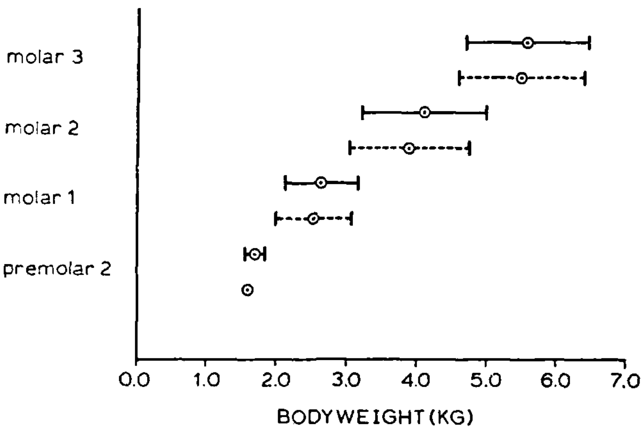

Figure 1 Dentition pattern versus bodyweight in wild caught *Presbytis cristata* (n = 169). Solid lines = upper jaw dashed lines = lower jaw.

However lung mites, *Pneumonyssus simicola*, were later found in pathological specimens from a number of animals.

Approximately 25% of the animals arrived with fresh minor lacerations, for which appropriate treatment was given, and approximately 15% displayed scars, healed fractures or evidence of other old injuries. Two females arrived pregnant but both aborted within 24 hours after arrival. A comparison of dentition patterns with body weight upon arrival is shown in Figure 1. Sex ratio and mean body weight of the animals collected for use in this study were similar to values reported by Walker, et a. (6) for *P. cristata* in Malaysia.

Hematological parameters of the animals used in this study displayed a wide range of values. This variance probably reflects the difference in state of health of individual animals upon arrival. White blood cell differential counts were similar to those reported by Walker, *et al.* (6), although the latter were determined for animals after 3 months in captivity. Erythrocyte associated values for *P. cristata* gradually increase with time in captivity, suggesting improvement of an anemic condition common among animals in the wild (6).

P. cristata are arboreal animals that avoid contact with humans, however the monkeys in this study were found to be naturally infected with several species of parasitic organisms common to man. In most cases, the short time between capture of the animals in the field and examination in the laboratory precluded acquisition of these infections from human animal trappers and handlers. Parasitological results from this study differed from those of Palmieri, *et al.* (7), however the latter study reported results from animals captured in a different geographical region of Indonesia. Arambulo, *et al.* (8) also reported high prevalence rates of *T. trichiura* and *Strongyloides* among wild caught *P. cristata* in Malaysia.

Culture of rectal swabs obtained on arrival detected pathogenic microorganisms in only a few animals. This could reflect a less than optimal isolation method rather than a lack of pathogens. However, with the increased human contact that accompanied captivity, the monkeys began experiencing frequent episodes of diarrhea. This was a serious problem that required constant vigilance and aggressive treatment with antibiotics and rehydration. A common and serious finding among new animals was respiratory infection, which if not aggressively treated was often fatal. A common etiology for this illness was not established, however the animals frequently responded well to trimethoprim-sulfamethoxazole or penicillin-streptomycin therapy. In many cases, it appeared probable that the animals' illness was exacerbated by the stress of captivity and handling.

Although procedures for locating ectoparasites on the animals were not optimal, the complete absence of findings suggests that in the wild state, the *P. cristata* used in our study did not carry heavy burdens of ectoparasites. However, lung mites were found at inflammatory foci in pathologic specimens from several monkeys, and may predispose their hosts to respiratory infections.

New animals presented with various minor injuries, particularly lacerations of the scalp, which we observed to result from stress behavior of striking the head against the cage and bites received from other captive monkeys.

Figure 1 shows mean body weights at which various dental patterns were observed. Although these data cannot establish a clear correlation between dental pattern and age of the animal, as has been established for *Papio* sp. (9), they represent the first step in producing such a correlation for the better understanding of this important yet fragile animal.

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The silvered leaf monkey, Presbytis cristata, is presented as an experimental animal model for human bancroftian filariasis. In this paper baseline physical, hematological and parasitological data were obtained from 169 wild caught P. cristata. Animals were found naturally infected with a variety of protozoan and helminthic parasites including human pathogens. Bacteriological findings at time of capture were minimal although with increased human contact, gastrointestinal, respiratory and other infections among the animals became a significant management problem. [illegible] Epidemiology, Reprints. [illegible]

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